CELLULAR SENSING DEVICES FOR ASSESSING CHEMICALS

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Abstract-**Several types of cellular biosensors have been developed for assessing chemicals, in which the cell responses to chemicals are transduced to the sensor outputs in various manners. The cell layer is fixed in the vicinity of the signal transducer. As an example, assessment of immunomodulating chemicals has been performed with a cellular biosensor based on electrochemical determination of the inducible nitrogen oxide synthase (iNOS) expression in a macrophage-like cell.**

1. INTRODUCTION

Biological effects of chemicals have been assessed in general by animal tests prior to clinical tests. Increasing efforts, however, have been concentrated on development of animal test alternatives due to public acceptance. Although it is not easy to establish the animal test alternatives, several alternatives have been proposed as a primary screening test. Of these, bioassay based on cultured cells seem promising, though it suffers from long and tedious procedure. To overcome the drawbacks of the conventional cell culture bioassays, we have developed a novel method for assessing chemicals with a cellular biosensing system, in which the cell responses to chemicals are directly transduced to the sensor output.

A living cell contains a whole set of intracellular information networks including gene information networks and signal transduction pathways. Molecular messengers such as hormones, cytokines and neurotransmitters are recognized by receptors embedded on the cell membrane surface, resulting in activation of the intracellular information networks mostly to modulate the gene information networks. The intracellular information networks might be purturbed by foreign chemical substances, which is resulted in cell responses. The effects of chemicals on the intracellular information networks may thus be assessed by measuring the cell responses.

2. ASSESSMENT OF CHEMICALS BY NO SYNTHASES EXPRESSION

In the intracellular information networks, nitrogen oxide (NO) plays an interesting role on modulating various physiological functions. A family of nitrogen oxide synthases, cNOS and iNOS, catalyze NO generation from L-arginine to L-citrulline. The constitutive isoform of cNOS is steadly expressed in neural and endothelial cells to work in a Ca^{2+}/calmoduline dependent manner. In contrast, the inducible isoform of iNOS is temporarily expressed, in macrophages, microglia, and astrocytes, in response to various stimulations. Two types of cellular sensing devices may be designed as followings;

1) cNOS-based Cellular Sensing Device

The cNOS-based cellular device quantitates NO generated enzymatically by cNOS in such cell as endothelial cells. As blood pressure, for instance, is regulated by released NO, the cellular device with cNOS is effective on assessing chemicals which concern with vascular relaxation. The cultured cells are layered on a polyion complex film under which an NO detecting electrode is fixed. Some chemicals are in contact with the surface of the cNOS-based cellular sensing device and may interact with the intracellular information networks to modulate the cNOS activity, which results in an increased or decreased release of NO. The chemicals are assessed as blood pressure regulating pharmaceuticals in relation to the cNOS-based cellular sensing device output.

2) iNOS-based Cellular Sensing Device

In a similar fashion to the cNOS-based cellular sensing devices, an iNOS-based cellular sensing device may be constructed. The iNOS expression is activated by such factors as immunomodulators, in which transcription of the iNOS gene is initiated. It takes some time

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Abstract

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to get the iNOS-based sensing device, because iNOS appears in a delayed time, although NO is released intensively.

3. RESPONSE CHARACTERISTICS

The macrophage-like cells (RAW264.7) were cultured on the NO detecting electrode coated with a polyion complex layer, to construct the cellular biosensing device. Released NO from cells was measured after LPS and IFN-γ were added into the system. The NO electrode could detect released NO in the same way of nitrite/nitrate detection, after RAW264.7 cells were stimulate with LPS and IFN-γ. However, using the method with the cellular biosensing system, it could obtain results faster than Griess' method. The measurement took about 1 hour with Griess' method, but by using the cellular biosensing system we shortened measuring time to about 600 ms. By culturing RAW264.7 cells on the NO sensor directly, it could detect NO directly and which make the measuring result more accurate and reliable. This novel cellular biosensing system made assessment of chemicals easy and practical.

By using this cellular biosensing system, the standard samples could be assessed as the drugs which had influence on iNOS cascade of the immune system, and many unknown drugs could be sorted as what had the effect on iNOS cascade or not.

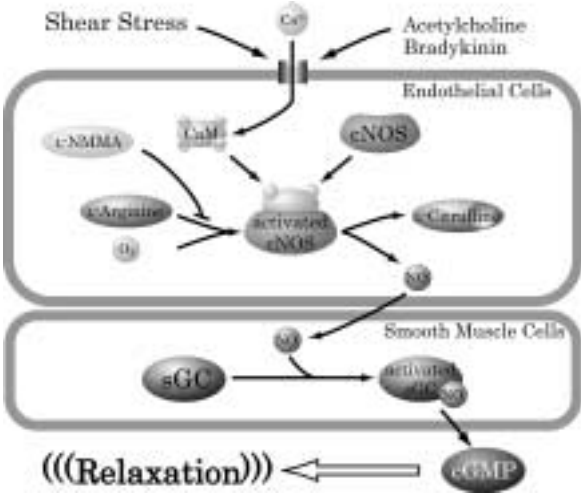

Fig. 1 Intracellular information networks linked with cNOS

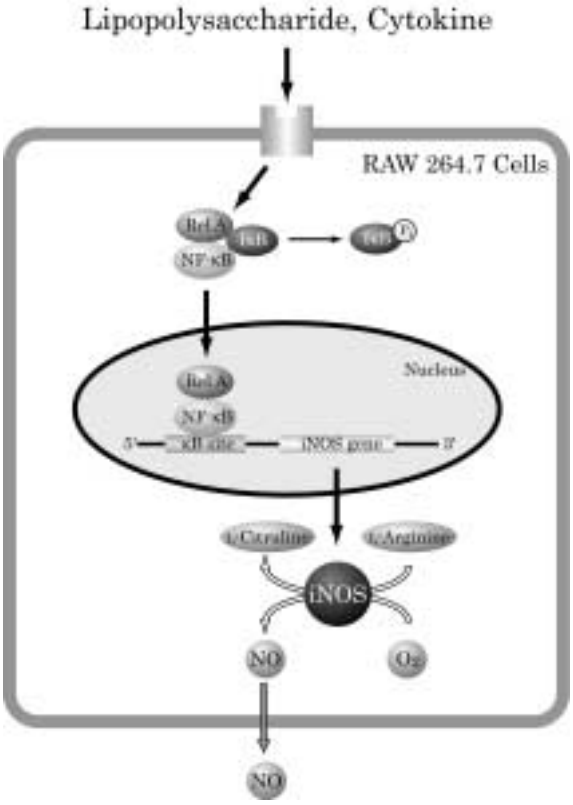

Fig. 2 Intracellular information networks linked with iNOS